Philip Siekevitz, who joined the Zamecnik group in 1949 as an NIH Fellow, adapted the techniques developed by Hogeboom et al. at Rockefeller to create a fraction that contained both mitochondria and microsomes in which he could study incorporation of labeled amino acids. When he added citric acid cycle intermediates, incorporation increased. If respiration were suppressed, he found adding ATP could foster incorporation (Siekevitz & Zamecnik, 1951; Siekevitz, 1952). These were important clues to the energetics of protein synthesis and offered support for Lipmann's conception.

Yet further enhancements in the fractionation procedure, especially use of the Potter-Elvehjem homogenizer, enabled Nancy Bucher (1953) in Zamecnik's laboratory to demonstrate incorporation of labeled acetate into cholesterol and subsequently into proteins. Using this preparation Zamecnik and Elizabeth Keller showed that it was the microsomal and supernatant fractions that were required for protein synthesis, and that they had to be supplemented by ATP and guanosinetriphosphate (Keller & Zamecnik, 1956). Then, in collaboration with Mahlon Hoagland, Zamecnik and Keller revealed that the ATP interacted with the amino acid, forming an amino acyl ~ AMP compound, in the soluble protein fraction (Hoagland, Keller, & Zamecnik, 1956; Hoagland, 1955). They construed this as *activating* the amino acid, thus enabling it to form a peptide bond with another amino acid. With this research, the Zamecnik group (a) secured the claim that Claude's microsome fraction was the locus of protein synthesis, and (b) demonstrated the dependency of these activities on energy made available by the mitochondrial system.

Integrating Morphology and Biochemistry

In 1952 Siekevitz left Zamecnik's group to go to Wisconsin to work on oxidative phosphorylation with Van Potter. Three years later, George Palade recruited him to the Rockefeller Institute. After Hogeboom and Schneider had left, the Rockefeller group had not had a researcher primarily trained in biochemistry and had focused their efforts on electron microscopy. Those efforts had paid off handsomely, but it was now necessary to figure out the operations performed by the differentiated components, especially the laminar membranes of the endoplasmic reticulum and the small particles that dotted their surfaces in many places. With Siekevitz providing biochemical expertise, Palade immediately set out to conduct an "integrated morphological and biochemical study" of microsomes, initially from liver and subsequently from pancreatic acinar cells. They examined a portion of the specimen at each step in the fractionation process under the electron microscope. In the homogenate they identified "'hollow' profiles comparable in size, shape, and number to